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Single molecule Raman spectroscopy using silver and gold nanoparticles

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Abstract : We discuss single molecule Raman spectroscopy based on the strongly enhanced Raman scattering signals which occur when a target molecule is attached to silver and gold colloidal nanoparticles. This phenomena known as surface-enhanced Raman scattering (SERS) exploits extremely large SERS enhancement factors of about fourteen orders of magnitude, with effective Raman cross sections reaching the level of fluorescence cross sections thus enabling a single molecule detection using surface-enhanced Raman spectrum The advantage of this method is that it not only detects a single molecule, it also simultaneously provides its structural fingerprint Moreover, SERS can be studied under electronic "nonresonant" conditions, which avoids photobleaching.

Detecting single molecules as well as identifying their chemical structures represents the ultimate limit in chemical analysis and is of great practical interest in many fields

This paper gives a brief introduction to single molecule SERS spectroscopy, describes nonresonant single molecule Raman experiments at near infrared excitation and discuss prospects and limitations of the method.

Keywords : SERS spectroscopy, single molecule detection, silver and gold nanoparticles

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1. Introduction

When light is reemitted by a molecule after optical excitation, in addition to fluorescence one can observe discrete spectral "lines", whose frequencies are uniquely shifted relative to the excitation frequency. The effect, called Raman scattering was first experimentally discovered in 1928 by Raman and almost simultaneously described by Mandelstam [1,2]. The spectrally shifted "Raman lines" occur due to the inelastic scattering of the incoming photons by the molecular vibrations. Therefore, the shifts of the scattered light gives direct information on the vibrational energies of the molecule and in this way, it provides something like its structural "fingerprint".

Over the past 75 years, Raman spectroscopy has been developed to become a valuable basic research tool and has become a powerful spectroscopic technique with many practical applications. However, until recently Raman spectroscopy has been considered to be more useful for structural analysis than for ultra-sensitive trace or even single molecule detection. The reason is the extremely small cross section for Raman scattering, typically ~10^{-30}-10^{-25} cm^2 per molecule, the larger values occurring only under favorable resonance Raman conditions. For comparison, fluorescence spectroscopy, which is widely used as a tool for the detection of single molecule exploits effective cross sections between 10^{-17} cm^2 and 10^{-16} cm^2 per molecule.

The small Raman cross sections require a large number of molecules to achieve adequate conversion rates from excitation laser photons to Raman photons, thereby precluding the use of Raman spectroscopy as a single molecule technique. Fortunately, the situation is dramatically improved if "surface-enhanced Raman scattering (SERS)" is used. About 50 years after the discovery of the Raman effect, the novel phenomenon of a strongly-increased Raman signals from molecules attached to metallic nanostructures attracted considerable attention from both basic and practical viewpoints [3-5]. As a spectroscopic tool, SERS has the potential to combine the sensitivity of fluorescence with the structural information content of Raman spectroscopy. Also, new improved methods resulted in SERS enhancement factors an unexpectedly large enhancement factors

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of at least fourteen orders of magnitude, demonstrating the Raman-scattering cross sections and fluorescence cross sections can be of the same order of magnitude [6] and thus detection of a single molecule with the high structural selectivity provided by its SERS spectrum can be realized [7-10].

Detecting single molecules and simultaneously identifying their chemical structures represents the ultimate limit in chemical analysis, and is of basic scientific and great practical interest in many fields. For example, monitoring molecules and molecular interactions at the single molecule level in cells or biological membranes and identifying single DNA fragments would be of great interest in the fields of biophysics and medicine. Furthermore, the observation of Raman scattering of an individual molecule is of basic scientific interest, as it provides insight into the intrinsic properties of the molecule and permits observations without any ensemble averaging.

In this article, we introduce single molecule Raman spectroscopy based on the strongly enhanced Raman scattering signals which occur when the target molecule is attached to silver and gold colloidal nanoparticles. Single molecule Raman experiments using near infrared nonresonant excitation are described and applications of ultrasensitive Raman spectroscopy are discussed.

2. The trail to single molecule Raman spectroscopy

In 1974 Fleischman, Hendra, and McQuillan observed unexpectedly high Raman signals from pyridine on a rough silver electrode [3]. They explained their experimental finding by an increase in surface area of the rough electrode resulting in an increase in the number of adsorbed molecules contributing to the Raman signal compared to observations from a "smooth" electrode. In 1977 Van Duyne and Jeanmaire, and, independently, Albrecht and Creighton confirmed the 1974 experiments but they inferred that the enormously strong Raman signal measured from pyridine on the rough silver electrode must be caused by a true enhancement of the Raman scattering efficiency itself, and that it cannot be explained only by an increase in the number of Raman scattering molecules [4, 5]. In 1979 Creighton, Blatchford and Albrecht measured enhanced Raman scattering from pyridine in aqueous silver and gold colloidal solutions [11]. These experiments showed that SERS is not so much a "surface effect" but is a "nanostructure effect" and provided the first clear experimental demonstration of the important role of plasmon resonances in SERS. On the other hand, from the very early days of the SERS effect it was pretty clear that there might not exist a single SERS mechanism and that different effects may contribute to the observed enhanced Raman signal in experiments when target molecules are adsorbed on very different so-called "SERS-active substrates". These SERS-active substrates are various metallic structures with sizes on the order of tens of nanometers. The most common types of SERS-active substrates exhibiting the largest enhancement effects are colloidal silver and gold particles in the range 10 to 100 nm size, "rough" silver or gold electrodes, or evaporated island films of these metals. For a general overview of the earlier days of SERS one may refer to excellent review articles by Otto [12] and Moskovits [13].

Estimated enhancement factors for the Raman signal started from modest factors of 10^3 to 10^5observed in the initial experiments. Many authors later could observe enhancement factors of about 10^{10} to 10^{11} for dye molecules in surface-enhanced resonance Raman experiments (SERRS) [14-26].

In 1987, we measured SERRS spectra from about 100 rhodamine 6G molecules in aggregated silver colloidal solution and concluded that in principle, even lower detection limits in SERS may be possible than in fluorescence spectroscopy [25]. The results were confirmed in 1995 where we collected SERRS signals from 60 rhodamine 6G molecules and discussed that single molecule Raman spectroscopy should be possible by further improving the experimental methods. [26].

In 1995, using a different approach, we could observe effective SERS cross sections from the population "pumping" of the first excited vibrational state by very strong Raman processes [6]. These experiments resulted in unexpectedly high nonresonant SERS enhancement factors of at least 10^{14}, amounting to nearly ten orders of magnitude higher than the first SERS enhancement factors. The pumping experiments also showed that only a very small number of molecules in the SERS sample are involved in the SERS process at such a high enhancement level. Based on such large SERS enhancement factors, we drastically reduced the number of molecules in the probed volume and measured Raman spectra from a single molecule using nonresonant near infrared excitation [7, 9, 10]. Nie and Emory improved the surface enhanced resonance Raman experiments to single nanoparticle-single molecule experiments [8].

3. A brief introduction to SERS

In SERS molecules are attached to metallic nanostructures resulting in a strongly enhanced Raman signal. Typical and very useful "nanostructures" are colloidal gold or silver particles as shown in Figure 1.

In principal, two effects might be operative in surface-enhanced Raman processes :

(i) The scattering takes place in the enhanced local optical fields of the metallic nanostructures (electromagnetic field enhancement).

(ii) a molecule in contact with a metal (nanostructure) exhibits a "new Raman processes" at a larger cross section than the Raman cross section of a free

molecule (chemical or electronic enhancement, first layer effect).

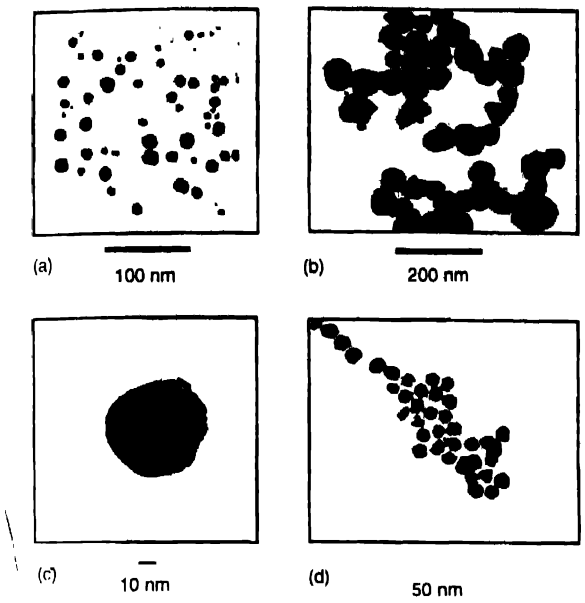
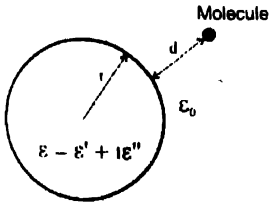


Figure 1. Typical SERS-active silver (top) and gold (below) colloidal clusters

Figure 2 shows a simplified schematic for understanding the concept of electromagnetic SERS enhancement using a small metal sphere with the complex dielectric constant $\varepsilon(\nu)$ in a surrounding medium with a dielectric constant ε_0. A molecule in the vicinity of the sphere (distance d) is exposed to a field E_M, which is the superposition of the incoming field E_0 and the field of a dipole E_{sp} induced in the metal sphere. Therefore, the effective optical field seen by the molecule is enhanced compared to the applied laser field. Figure 2 shows the formula for the field of the induced dipole. This field is particularly strong when the real part of $\varepsilon(\nu)$ is equal to $-2\varepsilon_0$. This condition corresponds to the resonant frequency of the surface plasmons in the metallic sphere. Additionally, for a strong electromagnetic enhancement,



$$E_m = E_0 + E_{sp}$$

E_{sp} : field of a point dipole in the center ofthe sphere

$$E_{sp} = r^3 \frac{\varepsilon - \varepsilon_0}{\varepsilon + 2\varepsilon_0} E_0 \frac{1}{(r+d)^3}$$

$$A(\nu) = \frac{E_M(\nu)}{E_0(\nu)}$$

$$G_{em}(\nu_L) = |A(\nu_L)|^2 \cdot |A(\nu_S)|^2$$

$$G_{em}(\nu_S) = \left|\frac{\varepsilon(\nu_L) - \varepsilon_0}{\varepsilon(\nu_L) + 2\varepsilon_0}\right|^2 \cdot \left|\frac{\varepsilon(\nu_S) - \varepsilon_0}{\varepsilon(\nu_S) + 2\varepsilon_0}\right|^2 \cdot \left(\frac{r}{r+d}\right)^{12}$$

Figure 2. Schematic of the concept of electromagnetic SERS enhancement (for explanation see text)

the imaginary part of the dielectric constant needs to be small. In an analogous fashion to the laser field, the scattered Stokes or anti Stokes field will be enhanced if it is in resonance with the surface plasmons of the metal sphere. Taking into account both field enhancing effects, the electromagnetic enhancement $G_{em}(\nu_S)$ for the Stokes power can be written as is shown in Figure 2. This formula based on a very simple model describes the most important properties and peculiarities of the electromagnetic SERS enhancement. It shows that the enhancement scales as the fourth power of the local field of the metallic nanostructure and that it is particularly strong when excitation and scattered fields are in resonance with the plasmon resonances. Electromagnetic SERS enhancement does not require direct contact between molecule and metal and the formula also describes the distance dependence of the electromagnetic enhancement, which is given by the decay of the field of a dipole over the distance ($[1/d]^3$) to the fourth power.

Electromagnetic field enhancement and surface plasmon resonance can also explain the initial discovery of surface-enhanced Raman scattering. This observation was possible because the blue and green emission lines of the Argon-Ion laser accidentally fell in the excitation range of surface plasmons due to the bumps on the electrochemically roughened silver electrode.

In general, the plasmon resonance frequency and also the size of the electromagnetic field enhancement factor depend on the size, shape, and of course, the material of the metallic nanoparticles and their environment [12, 13]. Maximum values for electromagnetic enhancement for isolated single colloidal silver and gold spheroids are on the order of 10^6 to 10^7. Theory predicts strong enhancement of electromagnetic fields for sharp features and large curvature regions, which may exist on silver and gold nanostructures. Also, closely spaced interacting particles can provide extra field enhancement, particularly near the gap sites between two particles in proximity. Electromagnetic enhancement factors up to 10^{11} have been estimated for the midpoint between two silver or gold spherical particles separated by a gap of 1 nm [29].

In many experiments, SERS-active substrates consist of a collection of silver or gold nanoparticles exhibiting fractal properties, such as colloidal clusters formed by aggregation of colloidal particles or metal island films. Figure 3 shows SERS-active colloidal silver particles in different aggregation stages. The broadening of the plasmon resonance when colloidal clusters are formed is demonstrated also in Figure 3, which shows the relatively narrow absorption spectrum of isolated silver colloidal particles centered at about 400 nm together with the broad extinction curve for silver colloidal clusters ranging from about 500 nm to 1000 nm. At a fractal cluster, the excitation is not distributed uniformly over the entire cluster but tends to be spatially localized in so-called "hot" areas [30, 31] Therefore, the surface of a fractal colloidal cluster structure shows a very

inhomogeneous field distribution. The size of the "hot areas" can be as small as a few nanometers, their locations depend strongly on the geometry of the fractal object and on the excitation wavelength and polarization of the optical fields. When optical excitation is localized in such small "hot spots", extremely large electromagnetic SERS enhancement (proportional to field enhancement to the fourth power!) up to 10^{12} was theoretically predicted for these areas.

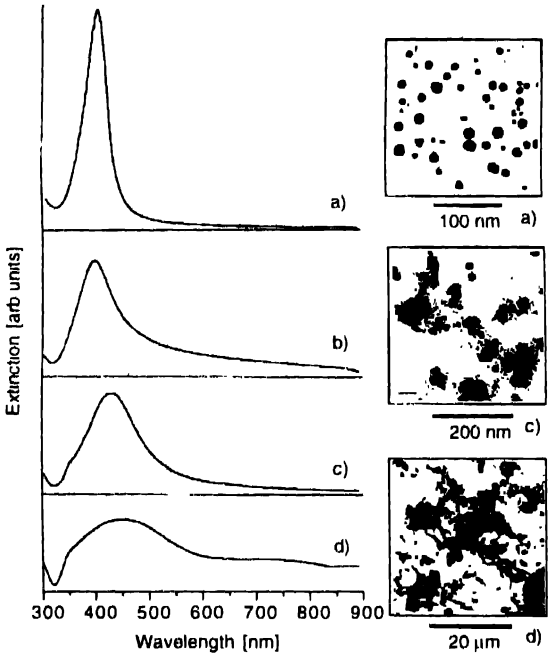


Figure 3. Electron microscope views and absorption/extinction curves of isolated silver colloidal particles and of silver colloidal clusters at different sizes

All the experimental findings described above given compelling evidence for an electromagnetic field enhancement. If SERS would be just an electromagnetic field enhancement effect, a strong SERS signal should exist for each molecule in the close enough vicinity of a silver or gold nanostructure. On the other hand, experimental observations such as dependence of the effect on the chemical nature of the molecule and a strong molecular selectivity provide clear indications for the existence of an (additional) "chemical" SERS enhancement. Other experimental observations, which hint to other mechanism(s) than electromagnetic field enhancement include SERS enhancement factors on electrodes, which depend on the electrode potential. Moreover, best electromagnetic SERS enhancement factors leave a gap of about two orders of magnitude to the best experimentally observed nonresonant SERS enhancement factors on the order of 10^{14}, which suggests the existence of additional enhancement mechanism(s) accounting for the missing factors.

Several different mechanisms are discussed as origin of a "chemical" or "electronic" SERS effect, sometimes also called first layer effect since the mechanisms require direct contact between molecule and metal.

For deeper discussion of the first layer effect we refer to excellent review articles of our colleagues [27, 28], please see also the article by Andreas Otto in this issue.

4. Single molecule SERS experiments

The size of the effective SERS cross section, or, in other words, the size of the SERS enhancement factor is a key question for application of SERS as tool for single molecule detection, since the effective cross section must be large enough for generating a detectable Raman signal from a single molecule. From single molecule fluorescence experiments one can conclude that effective cross sections for optical single molecule detection should be on the order of $10^{-16} - 10^{-17}$ cm^2 [32]. Assuming nonresonant Raman scattering cross sections on the order of 10^{-30} cm^2, single molecule SERS experiments require enhancement factors of $10^{13} - 10^{14}$.

This order of magnitude of the enhancement can be understood by a superposition of a factor 10^{12} electromagnetic SERS enhancement and a factor 100 chemical enhancement.

For colloidal silver and gold clusters such strong SERS enhancement factors exist in the near infrared (NIR) wavelength range. Fortunately, high-intensity NIR diode lasers are easily available, making this region also attractive for compact, low cost Raman instrumentation. Further, the development of low noise, high quantum efficiency multichannel detectors (charge-coupled device (CCD) arrays), combined with high-throughput single stage spectrographs used in combination with holographic laser rejection filters, has led to high sensitivity Raman spectrometers. In general, a state-of-the-art NIR Raman systems should have parameters which allow to perform single molecule SERS experiments.

SERS active substrates which provide a large enough enhancement factor and which are also compatible with single molecule methodologies are the most important requirement for single molecule SERS spectroscopy. As discussed in section 3, colloidal silver or gold particles, particularly their aggregates provide a very high SERS enhancement level. For single molecule detection in solution, small silver and gold colloidal clusters in sizes between about 100 and 1000 nm are very useful SERS-active substrates. At very low analyte concentrations of the target molecule (ca 10^{-11} M and lower), when the number of target molecules becomes comparable or smaller than the number of the colloidal clusters, no analyte induced cluster-cluster aggregation occurs, and the SERS spectra show a very good reproducibility. The smallest colloidal clusters we used in single molecule SERS experiments are between 150 - 300 nm in size, formed by only 10 - 30 individual colloids (see for example [9, 10]). Other authors have achieved single molecule sensitivity in SERS even for smaller silver clusters containing 2 - 5 colloidal particles [33, 34]. This is in agreement with theoretical estimates for two particles in close contact show particularly strong electromagnetic enhancement on sites between particles [29].

Studies on silver and gold colloidal cluster show that both metals are comparably good for single molecule Raman spectroscopy.

Figure 4 shows a schematic of a typical single molecule SERS experiment performed in silver or gold colloidal solution [7, 9,]. Spectra are excited by an argon-ion laser pumped cw Ti:sapphire laser operating at 830 nm with a power of about 100-200 mW at the sample. A microscope attachment is used for laser excitation and collection of the Raman scattered light. The analyte is provided as solution at concentrations smaller 10^{-11} M which is added to the solution of small silver or gold colloidal clusters. Concentration ratios of silver clusters and target molecules of at least 10 make it unlikely that more than one analyte molecule will be attached to the same colloidal cluster avoiding formation of aggregates of the target molecule on the surface. Addition of the analyte at such low concentration does not induce coagulation of the colloidal particles/colloidal clusters avoiding formation of larger clusters. The described procedure results in individual single molecules that are adsorbed on silver colloidal clusters. Analyte concentrations on the order of 10^{-12} to 10^{-14} M and probed volumes whose sizes are on the order of femtoliter to picoliter result in average numbers of one or fewer target molecules in the focus volume.

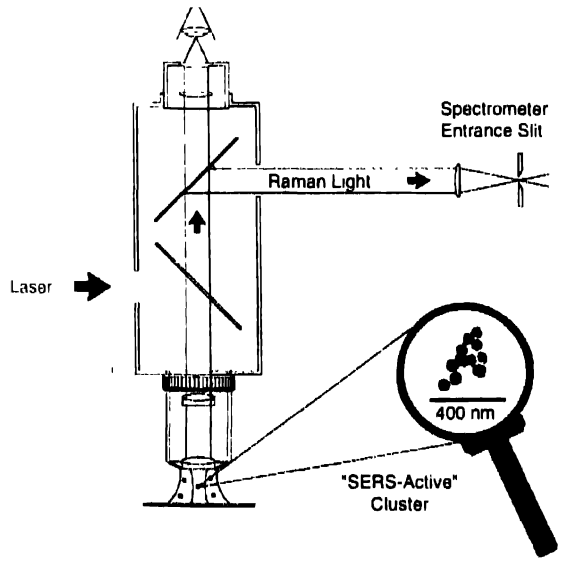


Figure 4. Schematic of a single molecule Raman experiment.

Brownian motion of single analyte molecule-loaded silver or gold clusters into and out of the probed volume results in strong statistical changes in the height of Raman signals measured from such a sample in time sequence. This is demonstrated in Figure 5 which shows typical unprocessed SERS spectra measured in time sequence from a sample with an average of 0.6 crystal violet molecules in the probed 30 pL volume [7]. Figure 6 displays the peak heights of the 1174 cm^{-1} crystal violet Raman line for the 100 SERS spectra (top), the background level of the colloidal solution with no analyte present (middle), and 100 measurement of the 1030 cm^{-1} Raman line of 3M methanol in colloidal silver solution (about 1014 molecules of methanol in the scattering volume) (below). The SERS signals appear in

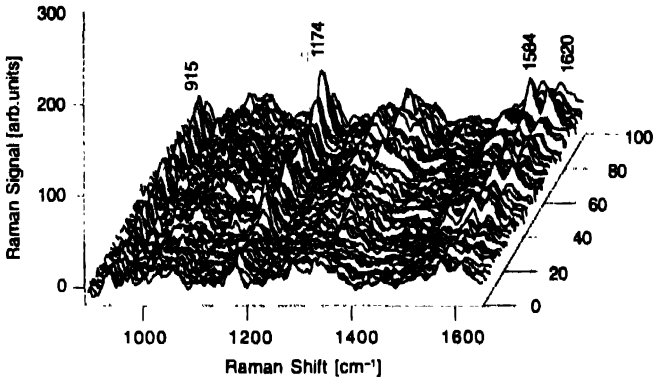


Figure 5. 100 single molecule SERS spectra of crystal violet on silver colloidal clusters. Collection time was one second for one spectrum, 830 nm excitation. Reprinted with permission from [7]. Copyright 1997 American Institute of Physics.

different power intervals, which can be assigned "0", "1", "2", and "3"-molecule events. The normal Raman signal of the 10^{14} methanol molecules appears at about the same level as the SERS signal of a single crystal violet molecule confirming an

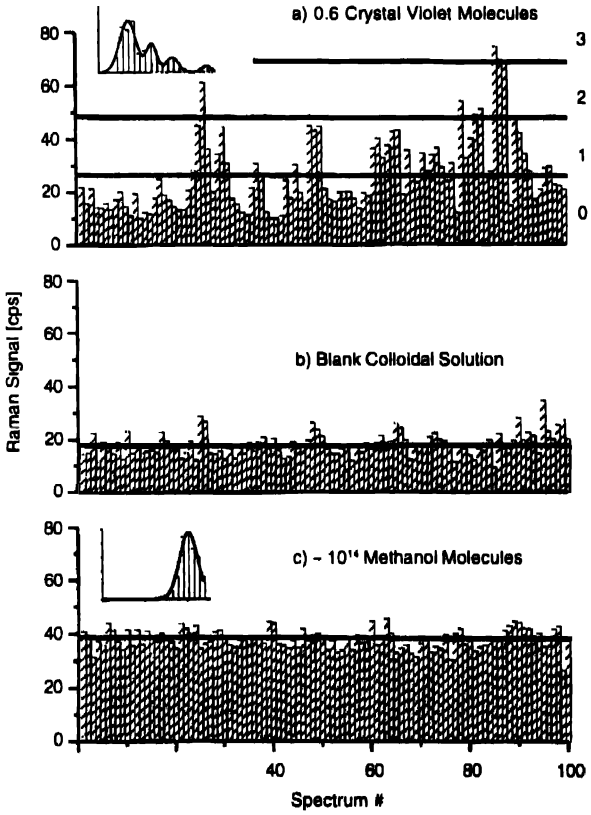


Figure 6. Peak heights of the 1174 cm^{-1} SERS line from an average of 0.6 crystal violet molecules (top), background signal (middle), peak heights of a Raman line measured from 1014 methanol molecules (below). The horizontal lines display the thresholds for one-, two- and three-molecule signals (top), the average background (middle), and the average 1014-molecule signal (below).

The inserts show the Poisson and Gaussian statistics of single molecule and many molecule Raman signals, respectively.

enhancement factor on the order of 10^{14}. The inserts in Figure 12 show the statistical distribution of the Raman and SERS signals. As expected, the methanol Raman signals collected in time sequence displays a Gaussian distribution. In contrast, the statistical distribution of the "0.6 molecules SERS signal " exhibits four relative maxima which are reasonably fit by the superposition of four Gaussian curves. The gradation of the areas of the four statistical peaks are roughly consistent with a Poisson distribution for an average number of 0.5 molecules. This reflects the probability to find 0, 1, 2 or 3 molecules in the scattering volume during the actual measurement. Comparing the measured Poisson distribution, which is in approximate agreement an average of 0.5, with the 0.6 molecule concentration/ volume estimate we conclude that about 80 % of molecules are detected by SERS. The change in the statistical distribution of the Raman signal from Gaussian to Poisson when the average number of target molecules in the scattering volume is one or fewer is evidence for single molecule detection by SERS.

Single molecule SERS spectra have also been measured at resonant laser excitation exploiting "molecular resonance enhancement" additionally to "surface enhancement". The mostly studied molecule is rhodamine 6G [8, 33, 34]. Brus and coworkers report extremely large surface-enhanced resonance Raman cross sections on the order of 10^{-14} cm^2 for this molecule on small colloidal silver clusters. SERRS spectra of single Hemoglobin molecules on silver colloidal dimers are reported in ref. [33].

In resonant single molecule experiments on fixed molecules, the authors observed fluctuations in the scattering signals including on/off behavior such as "blinking". Nie and Emory suggest that fluctuations in their SERS experiments arise from thermally activated diffusion (site to site hopping) of single adsorbed molecules on the particle surface.

A look at Figure 5 also shows fluctuations and changes in the solution SERS spectra collected in time sequence. Sometimes new Raman lines appear in a spectrum, which do not exist in the following trace. These spectral features are clearly not related to the target molecule. We ascribe these Raman lines to the surface enhanced spectra of impurities at the surface of the colloidal particles, which are, may be introduced in the chemical preparation process. The observation, that a SERS signal of a target molecule at extremely low concentration vanishes in a "background SERS signal" of impurities on the colloidal particles was discussed in our first studies of ultrasensitive SERS spectroscopy [25, 26]. The impurity spectra change because different colloidal clusters, which may loaded with different impurities move into the focal volume.

Perhaps the most surprising experimental observation in nonresonant single molecule Raman measurements in solution are the relatively well "quantized" signals for 1, 2 or 3 molecules suggesting relatively uniform enhancement despite the non-uniform shape and size (ca 10 - 50 nm) of the silver particles forming the clusters. This might be explained by "cluster-based enhancement", where a molecule "feels" an enhancement in the hot spots, which can be independent of the individual particles in the cluster, and also indepent of the size of the cluster one the cluster has exceeded a critical size. Moreover, the large field gradient at the "hot spot" might force the molecule to go to the hot spot and stay there.

It is also interesting to consider the maximum number of photons, which can be emitted by a molecule in fluorescence or Raman processes under saturation conditions. This number is inversely proportional to the lifetime of the excited states involved in the optical process. Due to the shorter vibrational relaxation times compared to electronic relaxation times, a molecule can go through more Raman cycles than fluorescence cycles per time interval Therefore, the number of Raman photons per unit time which can be emitted by a molecule under saturation conditions can be higher than the number of fluorescence photons by a factor of 10^2 to 10^3 [25].

5. Applications of ultrasensitive SERS spectroscopy

In general, SERS offers many interesting applications in many fields. Since its early days, the SERS effect was particularly appealing in the field of biophysics and biochemistry [35]. Most exciting for biophysical studies might be the trace analytical capabilities of SERS together with its high structural selectivity and the opportunity to measure Raman spectra from extremely small volumes. Particularly, single molecule capabilities open up exciting perspectives for SERS as a tool in laboratory medicine and for basic research in biophysics, where SERS can offer interesting new aspects compared to fluorescence, which is widely used as a single molecule spectroscopy tool in biophysics.

One of the most spectacular applications of single molecule SERS might appear in the field of rapid DNA sequencing using the Raman spectroscopic characterization of specific DNA fragments down to structurally sensitive detection of single bases without the use of fluorescent or radioactive labels [10] To detect and identify single DNA bases by fluorescence, they must be labeled by fluorescent dye molecules to achieve large enough fluorescence quantum yields and distinguishable spectral properties [32]. NIR-SERS provides a method for detecting and identifying a single DNA base, which does not require any labeling because it is based on the intrinsic surface-enhanced Raman scattering of the base. Effective Raman cross-sections of the order of 10^{-16} cm^2/molecule can be inferred for adenosine monophosphate (AMP) and for adenine on colloidal silver clusters. SERS spectra of adenine and adenosine monophosphate (AMP) are identical indicating sugar and phosphate bonds do not interfere with the strong SERS effect of adenine.

Due to the electromagnetic origin of the enhancement, it should be possible to achieve SERS cross sections for other

ases the same order of magnitude as for adenine when they are ttached to colloidal silver or gold clusters. The nucleotide bases how well-distinguished surface-enhanced Raman spectra, also hown in Figure 7b. Thus, after cleaving single native ucleotides from DNA or RNA strand into a medium containing olloidal silver clusters, for instance, into a flowing stream of olloidal solution or onto a moving surface with silver or gold luster structure, direct detection and identification of single native nucleotides should be possible due to unique SERS spectra of their bases (see Figure 7b).

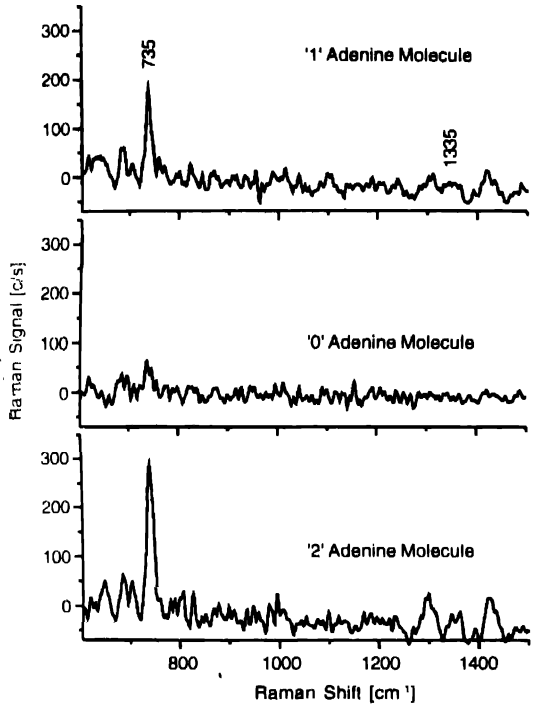


Figure 7a. SERS spectra of single adenine molecules in silver colloidal solution measured in one second collection time using 830 nm cw excitation (ca 100 mW).

Ultrasensitive surface-enhanced Raman measurements have been also performed inside single living cells. Colloidal gold particles (60 nm in size) that are deposited inside cells as "SERS-active nanostructures" result in strongly enhanced Raman signals of the native chemical constituents of the cells [36]. Particularly strong field enhancement can be observed when gold colloidal particles form colloidal clusters as it is shown in Figure 8. The strongly enhanced Raman signals allow Raman measurements of a single cell in the 400 - 1800 cm^{-1} range with 1 μm lateral resolution in relatively short collection times (1 second for one mapping point) using 3 - 5 mW near infrared excitation. Figure 8 shows typical unprocessed surface enhanced Raman spectra measured with 1 μm spot size within the 30 × 30 μm^2 area at different places of a cell monolayer incubated with colloidal gold. The one second collection time spectra show a very good signal to noise ratio allowing for the possibility of much shorter collection times. The SERS signal appears on the order of thousands of counts per second compared to signals of counts per second measured in "normal" Raman spectroscopy of single cells. SERS mapping over a cell monolayer with 1μm lateral resolution shows different Raman spectra at almost all places, reflecting the very inhomogeneous chemical constitution of the cells. The Raman lines can be assigned to native chemical constituents in the cell nucleus and cytoplasm, such as DNA, RNA, phenylalanine, tyrosine *etc.*

Our studies have shown, that colloidal gold supported Raman spectroscopy in living cells provides a tool for sensitive and structurally selective detection of native chemicals inside a cell and for monitoring their intracellular distributions. This might

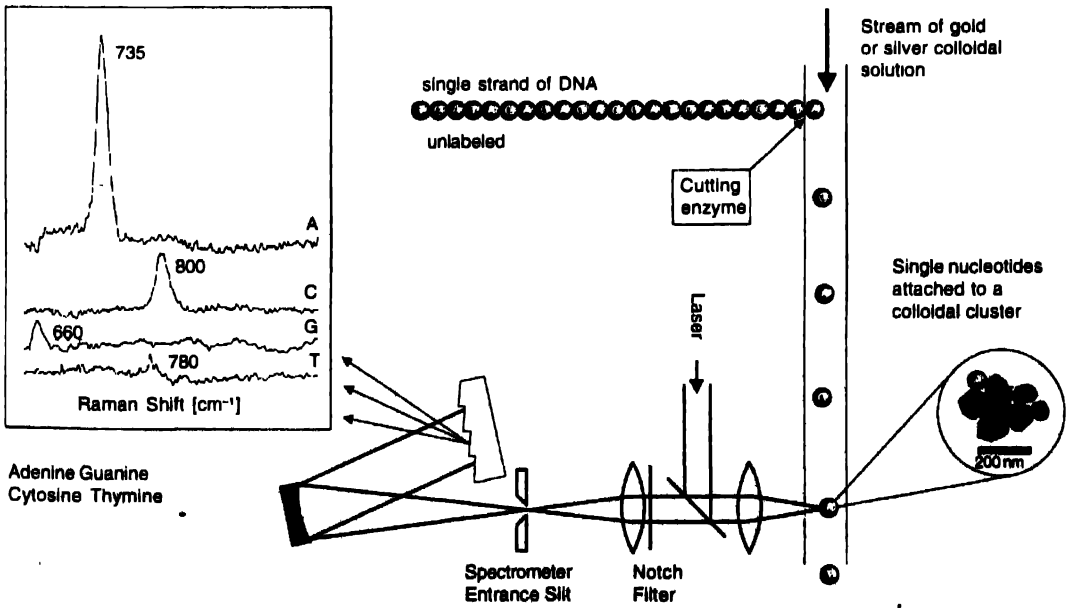


Figure 7b. Schematic of DNA sequencing based on the intrinsic Raman spectra of the four bases reprinted with permission from [35]. Copyright Institute of Physics.

open up exciting opportunities for cell biology and biomedical studies.

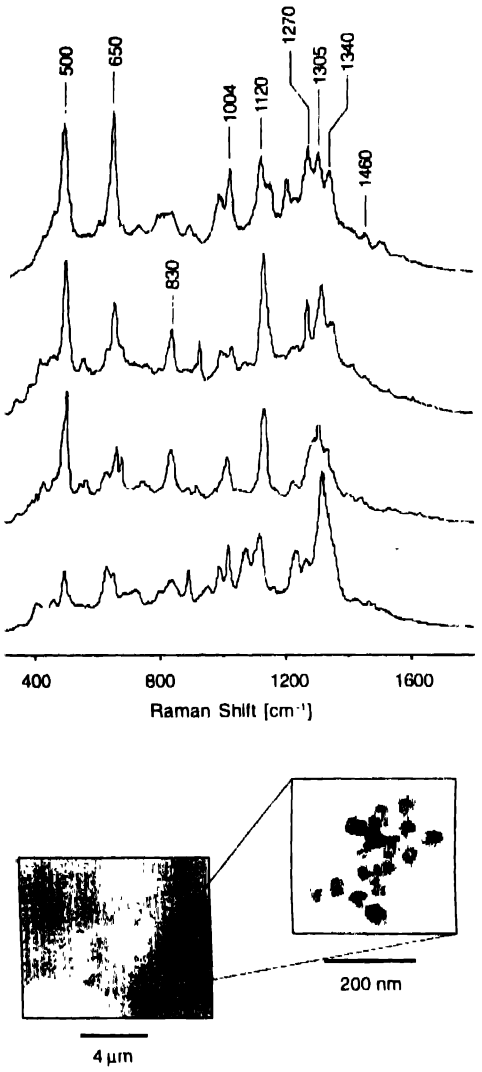


Figure 8. SERS spectra measured with 1 mm spot size from different places of a cell monolayer incubated with colloidal gold (top).

Electron micrographs of colloidal gold particles inside a cell monolayer. The higher magnification shows that the gold particles are aggregates of 60 nm colloidal spheres

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6. Summary

There is a strong interest in methods for detection and structural characterization of single molecules under ambient conditions. The fluorescence method provides ultra-high sensitivity but, particularly at room temperature, the amount of molecular information which can be obtained from the broad fluorescence bands is limited. The main advantage of Raman spectroscopy is its capability to provide rich information about molecular structure. In surface-enhanced Raman scattering effective Raman scattering cross sections can be enhanced up to fourteen orders of magnitude. This enhancement results in effective Raman cross sections comparable to, or even better than fluorescence cross sections. Therefore, as a spectroscopic tool, SERS has the potential to combine the sensitivity of fluorescence with the structural information content of Raman spectroscopy. Moreover, using SERS, non-fluorescent molecules such as nucleotides and amino acids might be detected and identified at the single-molecule level without fluorescence labeling.

Since SERS takes place in the local optical fields of metallic nanostructures, the lateral resolution of the technique is determined by the confinement of the local fields, which can be two orders of magnitude better than the diffraction limit [37].

In single molecule Raman experiments in solution we exploit SERS on silver and gold colloidal clusters using near infrared laser excitation. It should be noted, that near infrared SERS experiments are non-resonant to the electronic states of the analyte molecule and only vibrational energy is stored in the molecule.

Interesting aspects in applications of SERS come also from exploiting surface enhanced resonance Raman scattering (SERRS). In addition to the increased cross sections, resonance Raman scattering (RRS) has the advantage of higher specificity, since the resonance Raman spectrum is dominated by molecular vibrations, which are related to the part of the molecule, responsible for the appropriate resonant electronic transition SERRS opens interesting opportunities for large molecules, such as biomolecules, where it allows a selective vibrational probe of the chromophoric systems such as chlorophyll, pheophytin, carotenoids. As a further advantage, the fluorescence background, which can make RRS spectroscopy extremely difficult, has been quenched in many SERRS experiments by new nonradiative decay channels provided by the SERS-active metal.

Another useful capability of single molecule SERS comes from the potential of the method for providing information on molecules residing on surfaces and on surface and interface processes. For example, "SERS-active" silver or gold electrodes with a defined potential can be used as model environment for studying charge transfer transitions.

A comprehensive, quantitative understanding of the mechanisms of SERS, which should be achieved during the next few years, might be an important key for further development of SERS as single molecule tool.

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